Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010;363:1005-15. DOI: 10.1056/NEJMoa0907847.

Description of enrolment sites

Study participants were enrolled from geographically diverse populations with a high prevalence of TB, but differences in prevalence of HIV, smear negative TB and MDR TB. The Indian and Azerbaijan sites were known to have high MDR rates among a largely referral population with prior testing for TB in the periphery. TB DOTS centers used as enrolment sites in South Africa and Peru were primary health care settings and most of the pulmonary TB suspects from these sites had no prior testing.

1. **Lima, Peru**
   The Tropical Medicine Institute Alexander von Humboldt, Universidad Peruana Cayetano Heredia (UPCH) is located in northern Lima, Peru and is enrolling TB suspects from 30 primary health care DOTS treatment centers in surrounding shanty towns with a TB case notification rate of 192 per 100,000 inhabitants. The smear positivity rate in this setting is approximately 40%, whereas the rates of smear-negative, culture-positive (4%) and MDR patients are comparatively low. The HIV coinfection rate is <3%. The study was approved by the Institutional Review Board (IRB) of both, UPCH and the Ministry of Health DISA Lima Este.
2. **Cape Town, South Africa**
   The Institute for Infectious Diseases and Molecular Medicine, University of Cape Town (UCT) is situated in the Western Cape Province of South Africa (annual TB incidence 1037 per100 000 in 2005). Recruitment took place at a clinic at Khayelitsha, an informal settlement in Cape Town, where the annual TB incidence was 1622 per 100,000 in 2005. 30% of TB investigations in the region result in a positive smear or culture. The HIV co-infection rates are currently estimated at 77%. A recent MDR survey showed relatively low MDR rates of 4%. The study was approved by the IRB of UCT.
3. **Durban, South Africa**
   The site of the South African Medical Research Council (SAMRC) is situated in the suburb of Overport in the city of Durban, province of KwaZulu-Natal. Recruitment took place at 6 TB clinics within or surrounding Durban. Based on the provincial data, the TB case notification rate in the enrolment area is estimated to be 1094 per 100,000. MDR rates among new TB patients were found to be 4% in 2005. In a recent study, the HIV coinfection rate among TB cases was determined at 66%. The study was approved by the IRB of University of KwaZulu Natal and by the Scientific Committee of the Department of Health.
4. **Baku, Azerbaijan**
   The Special Treatment Institution (STI) is located in Baku, Azerbaijan, and screens all MDR/XDR suspected prisoners in Azerbaijan as well as all TB-suspected prisoners from the region, independent of whether they are suspected to have MDR or not. The TB prevalence among the screened population is high and 25% of culture positive patients (40% of patients at risk of drug resistance) are estimated to have MDR or XDR. The HIV coinfection rate is approximately 6%. Whereas study laboratories for all other sites were located within a 10 km distance to the enrolment clinics, sputum samples from STI were shipped at 4°C to the German National Reference Laboratory in Borstel for testing. The study was approved by the Technical Committee of Main Medical Department of Ministry of Justice.
5. **Mumbai, India**
   The P.D. Hinduja National Hospital and Medical Research Centre (Hinduja) is a premier tertiary care centre in central Mumbai, India. As a tertiary hospital, Hinduja receives mostly treatment failures and complicated TB cases. At least 50% of 9500 annual culture requests are usually MTB positive (most of them are also smear positive) and approximately 50% of positive cultures are MDR. The estimated HIV coinfection rate is 5%. The study was approved by the IRB of Hinduja Hospital and Technical Committee of Central TB Division of India.

Table 1: Comparison of the overall sensitivity of a single LJ culture, a single MGIT culture and a single, direct Xpert MTB/Rif test using the results of 3 smears and 4 cultures per patient as a reference standard.

Patient group	Single LJ*	Single MGIT*	Single, direct Xpert †
Smear-positive, Culture-positive	93.0% (1016/1092)	97.7% (1104/1130)	98.2% (551/561)
Smear-negative, Culture-positive	69.3% (205/296)	84.4% (276/327)	72.5% (124/171)
All Culture-positive	88.0% (1221/1388)	94.7% (1380/1457)	92.2% (675/732)

* Calculated as the proportion of individual cultures that agrees with the reference standard of 3 smears and 4 cultures. Contaminated cultures are excluded. Patients with two valid cultures appear in the calculation twice.

† Results are shown for the direct MTB/RIF test performed on unprocessed sputum. For details, see manuscript, table 2.

Table 2: Sensitivity and specificity of Xpert MTB/Rif compared to alternative molecular tests for tuberculosis case detection used routinely at 3 trial sites. For the site in Baku, Azerbaijan, BD ProbeTec™ ET MTB System (ProbeTec) was used as second molecular test and for the sites in Cape Town, South Africa and Mumbai, India, Roche Cobas®Amplicor MTB Assay (Amplicor) was used as a second molecular test*.

Site		Sensitivity in C+ patients			Specificity in Non-TB patients		
		MTB/RIF (pellet)	MTB/RIF (direct)	Second molecular test (pellet)	MTB/RIF (pellet)	MTB/RIF (direct)	Second molecular test (pellet)
Baku, Azerbaijan							
	%	82.1%	83.7%	83.9%	100%	98.6%	95.7%
	(Correct / total)	(119/145)	(123/147)	(125/149)	(68/68)	(68/69)	(67/70)
	[CI]	[75.0% - 87.5%]	[76.9% - 88.8%]	[77.2% - 88.9%]	[94.7% - 100%]	[92.2% - 99.7%]	[88.1% - 98.5%]
Cape Town, South Africa and Mumbai, India							
	%	94.0%	94.6%	86.8%	99.1%	99.5%	100.0%
	(Correct / total)	(312/332)	(315/333)	(270/311)	(221/223)	(220/221)	(223/223)
	[CI]	[90.9% - 96.1%]	[91.6% - 96.6%]	[82.6% - 90.1%]	[96.8% - 99.8%]	[97.5% - 99.9%]	[98.3% - 100.0%]

*****DNA extraction was done from NALC-NaOH treated sputum pellet as per package insert. MTB/RIF data are shown for testing of sputum pellet (pellet) and for testing of unprocessed sputum (direct).

Table 3: Sensitivity of Xpert MTB/Rif for 3 recruited patient populations: a) patients suspected to have PTB, b) patients suspected to have MDR-TB – not on TB therapy (patients with prior TB or MDR contacts), and c) patients suspected to have MDR-TB – with TB therapy (treatment failures). Sensitivity was not significantly different between the three groups (p=0.96; based on 2df logistic regression likelihood ratio test)*.

Rate at site and MTB/RIF sensitivity	Patients suspected of PTB	Patients suspected of MDR-TB; not on TB therapy	Patients suspected of MDR-TB; on TB therapy
Lima, Peru;			
% of patients (excluding indeterminates)	86.2 (275/319)	12.2 (39/319)	1.6 (5/319)
Sensitivity % (Correct / Culture-positive) [CI]	98.9% (183/185) [96.1% - 99.7%]	100.0% (22/22) [85.1% - 100.0%]	100.0% (4/4) [51.0% - 100.0%]
Baku, Azerbaijan;			
% of patients (excluding indeterminates)	45.4 (114/251)	48.2 (121/251)	6.4 (16/251)
Sensitivity % (Correct / Culture-positive) [CI]	96.3% (78/81) [89.7% - 98.7%]	98.1% (52/53) [90.1% - 99.7%]	93.3% (14/15) [70.2% - 98.8%]
Cape Town, South Africa;			
% of patients (excluding indeterminates)	60.5 (211/349)	34.4 (120/349)	5.2 (18/349)
Sensitivity % (Correct / Culture-positive) [CI]	96.7% (87/90) [90.7% - 98.9%]	93.0% (40/43) [81.4% - 97.6%]	100.0% (15/15) [79.6% - 100.0%]
Durban, South Africa;			
% of patients (excluding indeterminates)	81.2 (254/313)	16.6 (52/313)	2.2 (7/313)
Sensitivity % (Correct / Culture-positive) [CI]	93.3% (28/30) [78.7% - 98.2%]	100.0% (9/9) [70.1% - 100.0%]	100.0% (6/6) [61.0% - 100.0%]
Mumbai, India;			
% of patients (excluding indeterminates)	33.9 (78/230)	20.4 (47/230)	45.7 (105/230)
Sensitivity % (Correct / Culture-positive) [CI]	97.9% (47/48) [89.1% - 99.6%]	100.0% (37/37) [90.6% - 100.0%]	98.1% (101/103) [93.2% - 99.5%]
Total Sensitivity % (Correct / Culture-positive) [CI]	**97.5% (423/434) [95.5% - 98.6%]**	**97.6% (160/164) [93.9% - 99.0%]**	**97.9% (140/143) [94.0% - 99.3%]**

*Variables were indicators for patient groups (suspected PTB, suspected MDR not on therapy, suspected MDR on therapy) treating PTB suspects as the reference group. Results were similar (p>0.90) when adjusting for covariates including site and smear status.